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With the author's compliments.

Multiplication of Micro-organisms

by

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"On the Multiplication of Micro-organisms." By PERCY F. FRANKLAND, Ph.D., B.Sc., F.C.S., F.I.C., Assoc. Roy. Sch. Mines. Communicated by E. FRANKLAND, F.R.S. Received June 8, 1886.

In a previous communication "On the Removal of Micro-organisms from Water" ("Proc. Roy. Soc.," vol. 38 (1885), p. 379), I had occasion to point out the extraordinary rapidity with which micro-organisms may become multiplied even in ordinary distilled water. It was there shown that if a few drops of diluted urine-water be added to ordinary distilled water and kept in a sterilised bottle plugged with sterilised cotton-wool, the number of micro-organisms remaining suspended in the water became multiplied in the following manner:—

Number of hours.	Number of micro-organisms obtained from 1 c.c. of water.
0	1,073
6	6,028
24	7,262
48	48,100

These numbers represent the number of micro-organisms found in the upper layers of the water without agitating the contents of the bottle. More recently this subject has been touched upon by Dr. T. Leone ("Gazzetta Chimica Italiana," xv, 385), who found that the micro-organisms present in the water supplied to the city of Munich became multiplied in the course of five days from 5 per cubic centimetre to upwards of half a million in the same volume of water.

As this subject is one which is of much importance, from many points of view, I have carried out a number of experiments with the object of throwing some light upon these phenomena.

Methods of Experiment.

The number of micro-organisms present in the liquids employed was determined by means of Koch's method of plate cultivation with gelatine peptone. The apparatus used was substantially the same as that described in my previous paper (*loc. cit.*), a few modifications of the process having, however, been since then introduced. The sterile plates are now placed upon a levelled glass slab, resting upon a dish containing iced water, and covered with a glass shade, the latter being momentarily raised whilst the inoculated and fluid gelatine is poured upon the plate. With this arrangement the solidification of the gelatine is almost instantaneous, and the plate can be at once transferred to the moist chamber for incubation, thus enabling the preparation of a very much larger number of plates in a given time than was formerly possible, when the rate of solidification of the gelatine was dependent upon the temperature of the room. Instead of a solution of mercuric chloride in the moist chamber, I now simply employ sterilised distilled water, as I have found that the presence of the mercuric chloride in the moist chamber may exercise a prejudicial effect upon the full development of the colonies on the plate. When these modifications of the process are carried out with care in a room in which the distribution of dust is scrupulously avoided, the aërial contamination is altogether insignificant, but the process should in all cases be checked by pouring blank plates, in order to ascertain whether the precautions employed have been adequate or not. When, however, sufficient experience has been acquired in the management of the process, the preparation of these blank plates becomes rather a matter of form than of any practical importance, nevertheless when mixtures

of organisms and not pure cultivations are being dealt with, it is a check which should on no account be neglected.

Scope of the Experiments.

The bacteriological examination by plate cultivation of a large number of natural waters showed that practically all waters contain micro-organisms in greater or less abundance, but that the number found in such natural waters varies within exceedingly wide limits. Thus whilst the number found in the rivers Thames and Lea usually amounts to thousands in the cubic centimetre, that found in certain deep well-waters, which I have had under continual observation, rarely exceeds ten in the same volume. It appeared to me, therefore, of interest to study the further history of the organisms present in these waters of different origin and character.

In the second part of the paper will be found a description of a number of experiments made upon the power of multiplication possessed by certain pathogenic organisms, when placed under similar conditions.

I. *Experiments with Micro-organisms present in Natural Waters.*

The waters which were made the subject of study are those supplied by the eight Metropolitan Companies to London, and which for various reasons are well adapted for purposes of experiment. Firstly, they include waters derived from various sources, viz., from two different rivers—the Thames and Lea—and from deep wells in the chalk; secondly, their chemical composition is well known, and is made the subject of a very complete monthly investigation; and thirdly, their biological character has now been under periodical observation for eighteen months past.

Micro-organisms in Crude River-water.

Samples of unfiltered river-water (temp. 2° C.) collected in sterile stoppered bottles were submitted to plate cultivation the day after collection, whilst duplicate samples were allowed to stand at a temperature of about 20° C. for five days, and then similarly examined. The following results were obtained:—

Description.	Temperature.	Colonies found in 1 c.c.
River Thames at Hampton Ferry, 15th January, 1886	2° C.	—
Examined 16th January, 1886 ...	.. ....	45,392
Examined 20th January, 1886 ...	20°	35,790
River Lea at Chingford, 15th January, 1886	3°	—
Examined 16th January, 1886 ...	.. ....	39,307
Examined 20th January, 1886 ...	20°	63,488

Number of Colonies obtained from 1 c.c.

Description.	Feb. 19/86. Day of collection.	Feb. 20/86. Standing through a frosty night.	Feb. 20/86. Standing a night in the incubator, 35° C.	Feb. 27/86. Standing 7 days in diffused light at 20° C.	Feb. 27/86. Standing 7 days in darkness at 20° C.	Feb. 27/86. Standing 7 days in flask plugged with cotton wool, in diffused light at 20° C.	Mar. 8/86. Standing 16 days ditto (plugged flask), at 20° C.	Feb. 27/86. Standing 8 days in incubator at 35° C.	Mar. 8/86. Standing 16 days in incubator at 35° C.
Thames at Hampton, collected Feb. 19, 1886	15,800	18,155	665,280	..	..	..	..	{ 4,062 3,170 }	15,460
Lea at Chingford....	20,600	15,646	..	8,017	17,555	14,161	15,897	—	—

The above figures show that the organisms present in the crude river-waters undergo no material alteration in number when preserved in frosty weather for a period of twenty-four hours, but that when kept during the same period of time in the incubator (temp. 35° C.), the multiplication which takes place is altogether enormous. In those samples which were kept at the ordinary temperature of the air there is no instance of multiplication, but, on the contrary, there is on the whole a slight decrease, and in one case a considerable reduction; but by far the largest reduction was found in the samples which had been incubated for eight days, the rapid increase in numbers which at first takes place on exposure to the temperature of the incubator being apparently followed by a correspondingly rapid decline.

Thus in the one case there is a slight diminution in the number, and in the other case increase; but in neither is the alteration very considerable. Similar samples collected on the 19th February, 1886, were examined after exposure to various conditions, as specified on p. 528.

Further experiments were made with a view of testing this behaviour of the micro-organisms present in river-water. The results obtained were as follows:—

Number of Colonies obtained from 1 c.c.

Description.	April 13/86. On day of collection, temp. 8° C.	April 15/86. Standing in dark at 20° C. for 2 days. (Same bottle.)	April 17/86. Standing in dark at 20° C. for 4 days. (Same bottle.)
River Thames at Hampton	12,250	4,386	2,018
River Lea at Chingford...	7,300	2,148	1,286

Thus in both cases there was a marked reduction in the micro-organisms after storing for two and four days respectively.

From the experiments made with these river-waters, it would appear that there is a decided tendency for the micro-organisms which they contain to become reduced in number when the waters are kept at the ordinary temperature of the air (20° C.), whilst the numbers may be temporarily enormously increased by exposing them to an incubating temperature.

A very large number of experiments was also made with the filtered river-waters, as supplied to the metropolis. These waters have substantially the same chemical composition as the rivers from which they are derived, but the number of micro-organisms which they contain at the time of collection rarely exceeds on the average 5 per cent. of that present in the raw river-waters. The results obtained are recorded in the following tables:—

Number of Colonies obtained from 1 c.c.

Description.	Jan. 16/86. Day after collection, temp. about 3° C.	Jan. 20/86. Standing 5 days in dark at 20° C.	Jan. 23/86. Standing 7 days in refrigerator.	Feb. 12/86. Standing 27 days at 20° C. in dark.	Mar. 2/86. Standing 45 days at 20° C. in dark.
Filtered River Waters.					
Chelsea	159	lost	11,437	2,923	—
West Middlesex	180	lost	2,063	312	76
Southwark	2,270	5,285	8,311	1,323	—
Grand Junction	4,894	15,950	14,965	3,757	—
Lambeth	2,587	6,980	6,774	2,749	—
New River	363	lost	750	3,132	43
East London	224	lost	3,394	947	410
Average	1,525	9,405	6,813	2,163	176

Experiments were also made in order to ascertain the extent of multiplication taking place when samples of the filtered river-waters are exposed for twenty-four hours respectively in frosty weather, and in the incubator. The results obtained are comparable with those already recorded for the crude river-waters which were similarly exposed.

Number of Colonies obtained from 1 c.c.

Description.	Feb. 23/86. Day of collection.	Feb. 24/86. Standing through frosty night.	Feb. 24/86. Standing through night in incubator.
Filtered River Waters.			
Chelsea	305	305	62,483
West Middlesex	80	112	3,580
Southwark	284	360	1,954
Grand Junction	208	263	22,842
Lambeth	265	314	44,289
New River	74	107	8,892
East London	252	633	7,152
Average	210	299	21,599

Thus the micro-organisms in the filtered river-waters, on standing for twenty-four hours, even in cold weather, undergo a slight although distinct multiplication, thus bearing out the observations made with the same waters when kept in the refrigerator for a week. It is

further seen that when allowed to stand at a temperature of 20° C., the multiplication which takes place in the course of a few days is generally very considerable indeed, whilst at the temperature of the incubator (35° C.) the multiplication, even in the course of a single night, is enormous. On the other hand, when the storage is continued for a sufficient length of time, the number of micro-organisms diminishes, and may become reduced below that which was present at the outset. It would appear, however, that the micro-organisms in these *filtered* waters become multiplied at 20° C. with far greater rapidity than those in the *unfiltered* waters, which, as already pointed out, have rather a tendency to become diminished, unless raised to the temperature of the incubator.

It now became a matter of interest to compare with the above the power of multiplication which is possessed by the micro-organisms found in deep well-waters. Similar experiments were, therefore, made with the waters obtained from one of the wells in the chalk at Deptford, belonging to the Kent Company.

Number of Colonies obtained from 1 c.c.

Description.	Jan. 27/86. Day after collection.	Jan. 30/86. Standing 3 days in refrigerator, temp. several degrees above 0°.	Jan. 30/86. Standing 3 days, at 20° C. (dark).	Feb. 12/86. Standing 16 days at 20° C. (dark).
Kent well ...	96	163	178,379	51,843

On comparing these results with those previously referred to, it will be seen that whilst these organisms in the deep well-water have but little tendency to multiply in the cold, the multiplication at 20° C. is far in excess of anything observed in the case of the river-waters.

Experiments were also made to ascertain the influence of the temperature of the incubator upon the organisms in these well-waters.

Number of Colonies obtained from 1 c.c.

Description.	Feb. 23/86. Day of collection.	Feb. 24/86. Standing through frosty night.	Feb. 24/86. Standing 1 night in incubator, 36—39° C.
Kent well	5	18	743

Again, in another experiment, the following results were obtained:—

Number of Colonies obtained from 1 c.c.

Description.	Apr. 14/86. Day of collection.	Apr. 15/86. Standing 1 day, at 20° C.	Apr. 17/86. Standing 3 days, at 20° C.
Kent well.....	7	21	495,000

These tables show the enormous capacity for multiplication which is possessed by the micro-organisms present in this deep well-water. This is the more surprising, at first sight, when it is borne in mind that this water contains the merest trace of organic matter. It must, however, be remembered that this water is at the outset almost wholly free from micro-organisms, and that it has never before been inhabited by such living matters; it is only reasonable to infer, therefore, that those of its ingredients which are capable of nourishing the particular micro-organisms which flourish in it are wholly untouched, whilst in the case of the river-waters, the most available food supply must have been largely explored by the numerous generations of micro-organisms which have inhabited them. It should also be mentioned that the number of different varieties of micro-organisms is far greater in the case of the river-waters than in the deep well-water, as is at once evident to the naked eye on inspection of a plate cultivation, the deep well-water plates having generally the appearance of a pure culture; in the latter case, then, the organisms present will probably have a freer field for multiplication than in the presence of competitors, some of which may not improbably give rise to products which are hostile to others. This would also explain the greater capacity for multiplication which we find, as indicated above, in the filtered as compared with the unfiltered river-waters. By the process of filtration the number of different varieties of micro-organisms is largely reduced, as is at once seen by the inspection of the plate cultivations, and those varieties which remain have apparently a more favourable opportunity for reproduction than in the presence of more numerous varieties.

II. *The Multiplication of Pathogenic Micro-organisms.*

The remarkable phenomenon of multiplication which is exhibited by the micro-organisms found in most natural waters obviously leads us to a consideration of the behaviour of parasitic micro-organisms when accidentally introduced, as they frequently must be, into waters of different composition.

In studying the behaviour of parasitic micro-organisms under these conditions, I have selected three forms which are recognisable with particular facility, owing to the highly characteristic appearances to which they give rise when cultivated in the gelatine-peptone medium.

These forms are—

1. Bacillus pyocyaneus.
2. Finkler-Prior's Comma Spirillum.
3. Koch's Comma Spirillum.

The pure cultivations of these organisms were obtained directly, in the case of the first two mentioned, and indirectly, in the case of the third, from the laboratory of the Hygienic Institute of Berlin.

1. *Appearance of the Bacillus pyocyaneus in Gelatine-tube Cultivations.*

At 20° C., within twenty-four hours of inoculation, the path of the needle is indicated by incipient liquefaction at the surface, which as growth proceeds undergoes funnel-shaped depression, the fringe of which soon exhibits blue-green fluorescence. The diameter of the liquefied portion increases until the whole width of the tube is affected, the downward extension of liquefaction proceeding in the form of an inverted cone, in the apex of which a flesh-coloured precipitate is formed. The blue-green fluorescence is seen only on and near the surface. Ultimately the whole of the gelatine becomes liquid, and of a dirty green colour.

Appearance of the Bacillus pyocyaneus in Gelatine-plate Cultivations.

At 20° C. the colonies are generally distinctly visible on the second day as small opaque light green disks, the whole plate presenting a blue-green fluorescence when viewed by reflected light. The size of the colonies is dependent upon the number present on the plate, being exceedingly minute when the latter is densely crowded, and reaching several millimetres in diameter when only a few are scattered over the surface. The amount of liquefaction also varies inversely as the number of colonies, but even after considerable liquefaction has taken place over the surface of the gelatine, the individual colonies remain perfectly distinct, and do not become confluent, and so lose their identity, as is the case with many liquefying organisms. Not unfrequently, especially when there are comparatively few organisms on the plate, those situated on the surface give rise to a remarkable and very beautiful flocculent expansion of irregular contour and sometimes several millimetres in diameter.

The plates possess a highly characteristic and very nauseous odour, which is at once apparent on opening a dish in which they have been developed. Viewed under the low power of the microscope each

colony when young has a very characteristic appearance, resembling a spherical or ovoid sea-urchin covered with spines, and light brown or gray in colour.

Magnifying power nearly 1000.

Magnifying power about 100.

2. *Appearance of the Finkler-Prior's Comma Spirillum in Gelatine-tube Cultivations.*

The growth of this organism very much resembles that of the *Bacillus pyocyaneus*, only that no colouring-matter is produced. The liquefaction, which is at first funnel-shaped, rapidly extends across the whole tube, and the downward extension into the solid gelatine becomes filled with a plug of a yellowish viscid material. In the course of a few weeks the whole gelatine, or at least as far as the needle at the time of inoculation has reached, becomes liquid, the yellowish precipitate resting on the bottom, but the liquefied portion also remains turbid.

Appearance of the Finkler-Prior's Comma Spirillum in Gelatine-plate Cultivations.

The plate cultivations of this organism are very characteristic. The colonies making their appearance already on the first or second day as small milky white disks, which increasing in size cause rapid liquefaction of the gelatine, the depressions being filled with a white fluid resembling thin milk. If the centres are at all closely approximated they very soon become confluent, and lose their identity, the whole plate becoming fluid.

Viewed under the low power of the microscope the colonies appear as brownish very finely granular disks, with a highly refracting edge, which is also granular and not perfectly sharp.

Magnifying power nearly 1000.

Magnifying power about 100.

3. *Appearance of Koch's Comma Spirillum in Gelatine-tube Cultivations.*

The growth of this organism in gelatine is slower than that of the other two. At 20° C., within a day or two of inoculation, the path of

the needle becomes visible as a fine thread, which sooner or later is followed by a conical depression at the surface; this depression is filled with air at its wider upper extremity, and slowly increases in width; as growth proceeds the track of the needle becomes thicker, and the air-cavity at the surface becomes partially filled with clear liquid, at the bottom of which there is a whitish somewhat flocculent precipitate.

The growth in peptone-broth is very rapid, the liquid becomes turbid, and on the surface there is formed a tough pellicle which increases in thickness, whilst the subjacent liquid is again clarified.

Appearance of Koch's Comma Spirillum in Gelatine-plate Cultivations.

The plate cultivations exhibit in the course of two or three days minute whitish spots, each situated at the bottom of a small depression on the surface. The colonies remain small, but when very numerous the substance of the gelatine becomes largely liquefied.

Magnifying power nearly 1000.

Magnifying power about 100.

Under the low power of the microscope the colonies present the appearance of somewhat irregular disks, with a more or less irregular and jagged edge, the interior of the disk being filled with coarsely granular matter.

Multiplication Experiments with Specific Organisms.

The pure cultivations of the three specific organisms mentioned above were introduced into the experimental waters in the following manner, so as to secure a degree of attenuation which admitted of satisfactory examination by plate cultivation.

One or more needlefuls of the cultivation are first inoculated into about 50 c.c. of sterilised distilled water contained in a sterile stoppered bottle; this first attenuation is then well shaken, so as to distribute the introduced organisms evenly throughout. From this first attenuation a certain number of drops are withdrawn by means of a sterilised pipette, and introduced into the sterilised experimental water contained in another sterile stoppered bottle. The latter is then in turn well shaken, and the number of organisms in a given volume determined by plate cultivation. This second attenuation is then exposed to any desired conditions of temperature, &c., and the number of organisms determined in it at suitable intervals of time.

Experiments with the Bacillus pyocyaneus.

A large number of experiments were made upon the vitality of this organism when introduced into various kinds of water. The results of these experiments show that on introducing this organism into purer water, such as distilled water, deep well-water, or filtered Thames water, in the first instance a greater or less reduction in the number of organisms capable of development often takes place, but sooner or later a considerable increase in their numbers generally follows, and only in very exceptional cases was the complete destruction of their vitality observed. On the other hand, when the same organism is introduced into sewage a rapid and large multiplication was observed in every case. From a number of bottles of distilled water which were inoculated with the *Bacillus pyocyaneus* in the manner described above, the following number of colonies were obtained.

Bacillus pyocyaneus in Distilled Water.

Number of Colonies obtained from 1 c.c.

	Day of preparation.	2nd Day.	3rd Day.	7th Day.	20th Day.	53rd Day.
No. 1 ..	6,100	203	—	—	276	100,000
„ 2 ..	6,800	368 (Incub.)	—	—	13,400 (Incub.)	69,000
„ 3 ..	15,400	1,557 (Incub.)	—	—	69,600 (Incub.)	249,000
„ 4 ..	3,200	—	6,200 (Incub.)	96,000 (Incub.)	—	—
„ 5 ..	3,200	—	0	19	—	—
„ 6 ..	114,000	—	—	—	38,000	—
„ 7 ..	165,000	—	—	0 (Incub.)	—	—
„ 8 ..	165,000	—	—	0	—	—

Note.—"Incub." means that the sample above was kept in the incubator at 35° C.

Bacillus pyocyaneus in Filtered Thames Water.

Number of Colonies obtained from 1 c.c.

	Day of preparation.	3rd Day.
No. 1	3,900	Innumerable.
No. 2	3,900	Innumerable (incub.).

Bacillus pyocyaneus in Deep Well-water.

Number of Colonics found in 1 c.c.

	Day of preparation.	3rd Day.	5th Day.	8th Day.
No. 1	3,900	Innumerable. (Incub.)	—	—
„ 2	3,900	Innumerable.	—	—
„ 3	269,000	—	432,000 (Incub.)	395,000 (Incub.)
„ 4	269,000	—	422,000	Innumerable.
„ 5	262,000	—	851 (Incub.)	87 (Incub.)
„ 6	262,000	—	195,000	227,000
„ 7	10,000	0	—	0
„ 8	10,000	0	—	0
„ 9	10,000	0 (Incub.)	—	0 (Incub.)

Bacillus pyocyaneus in Sewage.

Number of Colonies found in 1 c.c.

	Day of preparation.	3rd Day.	8th Day.	18th Day.
No. 1	29,000	Innumerable. (Incub.)	Innumerable. (Incub.)	547,000 (Incub.)
„ 2	29,000	90,500	Innumerable.	Innumerable.
„ 3	29,000	116,500	Innumerable.	Innumerable.

The great vitality of this organism is well illustrated by experiments No. 1, 2, and 3, with distilled water, in which after fifty-three days there were in each case a great many more colonies obtained than at the outset, although in each case the number was greatly reduced immediately after the inoculation. For experiment No. 5, with distilled water, the number of organisms was in the first instance so largely reduced that on the third day there were no organisms demonstrable even in a whole cubic centimetre, and even on the seventh day the number had only risen to nineteen in that volume. The corresponding sample (No. 4) placed in the incubator multiplied much more rapidly, showing 6200 colonies on the third day and 96,000 on the seventh in 1 c.c.

In the deep well and filtered Thames waters there is abundant evidence of extensive multiplication, the only cases (No. 7, 8, and 9)

in which the organisms disappeared being all taken from one and the same bottle. In the sewage again the multiplication of the organism was in each case very great.

Experiments with Koch's Comma Spirillum.

The first experiments were made with comma Spirilla from feeble cultivations in gelatine. In these cultivations the organisms appeared to grow with difficulty, producing little or no liquefaction, but only a deep depression on the surface of the gelatine at the point of inoculation. On subsequently inoculating into broth a powerful growth was obtained, and from this again a strong growth in gelatine was procured. It was with comma Spirilla from this broth cultivation that the subsequent experiments were made. When the comma Spirilla from the weak cultivations were introduced into various waters, including sewage, their presence was, with one exception, no longer demonstrable after the first day, whilst the waters inoculated from the broth cultivation not only yielded colonies of comma Spirilla on plate cultivation after seven days, but during this time slight multiplication was exhibited in the potable water, and very extensive multiplication in the sewage. Thus—

Comma Spirillum (from a weak cultivation in Gelatine) in Distilled Water.

Number of Colonies obtained from 1 c.c.

	Day of preparation.	2nd Day.	3rd Day.	5th Day.	6th Day.	8th Day.	11th Day.	15th Day.	19th Day.
No. 1 .	100	0	—	0	0	0	0	0	—
„ 2 .	10	—	—	0	0	0	0	0	—
„ 3 .	52	0 (Incub.)	—	0 (Incub.)	0 (Incub.)	0 (Incub.)	0 (Incub.)	0 (Incub.)	—
„ 4 .	4,000	—	—	0	—	—	—	—	—
„ 5 .	2,800	—	0	—	—	—	—	—	—
„ 6* .	—	—	—	2,213	286	498	4	25	0

* In this case the comma Spirilla were introduced into the experimental bottle *from the gelatine cultivation direct*, so that an appreciable quantity of the gelatine peptone must have been introduced at the same time; it is not, therefore, comparable with the preceding five experiments.

Comma Spirillum (from weak cultivation in Gelatine) in Sewage and Deep Well-water.

Number of Colonies obtained from 1 c.c.

		Day of preparation.	2nd Day.	4th Day.
No. 1	Sewage	525	0 (Incub.)	0 (Incub.)
„ 2	Sewage	525	0	0
„ 3	Sewage	525	0	0
„ 4	Deep well water	395	0 (Incub.)	0 (Incub.)
„ 5	Deep well water	395	0	0
„ 6	Deep well water	395	0	0

These experiments show that in every case the comma Spirilla from this *weak cultivation* lost their vitality in twenty-four hours when introduced into these dilute media. The one case (No. 6 in distilled water) in which they were subsequently demonstrable, differed from the others, inasmuch as this was the bottle in which the cultivation was first attenuated, in fact Nos. 1, 2, and 3 were prepared by diluting No. 6 about 300 times. But even in this first attenuation the vitality of the organism is seen to be destroyed within nineteen days, and similarly the first attenuation, from which the six last experimental bottles above were inoculated, was also examined after nine days, and the comma Spirilla no longer found demonstrable.

In the following experiments the comma Spirilla employed were taken from a vigorous cultivation in peptone-broth, the results obtained being as follows:—

Comma Spirillum (from vigorous Broth Cultivation) in Deep Well-water, Sewa and Filtered Thames Water.

Number of Colonies obtained from 1 c c.

		Day of preparation.	2nd Day.	5th Day.	6th Day.	9th Day.	11th Day.	17th Day.	29th Day
No. 1	Deep well	5,750	0 (Incub.)	0 (Incub.)	0 (Incub.)	—	0 (Incub.)	—	—
„ 2		5,750	0	0	0	—	0	—	—
„ 3	Sewage ..	4,750	Innum. (Incub.)	Innum. (Incub.)	Innum. (Incub.)	—	96,000 (Incub.)	—	—
„ 4		4,750	60,000	Innum.	Innum.	—	Innum.	—	—
„ 5	Deep well	456	18 (Incub.)	1,225 (Incub.)	—	147 (Incub.)	—	0 (Incub.)	0 (Incu
„ 6		456	57	3,834	—	1,232	—	0	0
„ 7	Sewage ..	300	Innum. (Incub.)	Innum. (Incub.)	—	Innum. (Incub.)	—	128,000 (Incub.)	56,0((Incu
„ 8		300	19,000	Innum.	—	Innum.	—	Innum.	Innu
„ 9	Filtered Thames	—	188 (Incub.)	0 (Incub.)	0 (Incub.)	0 (Incub.)	—	—	—
„ 10		—	63	313	480	173	—	—	—

Thus the comma Spirilla from the vigorous cultivation in broth was found in every case to flourish in sewage; on the other hand, in deep well-water their vitality was lost in Nos. 1 and 2, whilst in Nos. 5 and 6 they were still demonstrable on the ninth day, having in the interim undergone considerable reduction in the first instance, and then multiplied, although incomparably less than in the sewage experiments.

The first attenuation (in distilled water) from which Nos. 1, 2, 3, and 4 above were inoculated was also examined on the ninth day, and 15,650 colonies obtained from the cubic centimetre. Thus, although still alive, the reduction in number must have been very great, as originally this attenuation must have contained upwards of 1 million per cubic centimetre.

Dr. Koch (Second Cholera Conference, May, 1885, "Brit. Med. Journ.," January 9, 1886) mentions that Nicati and Rietsch have demonstrated the vitality of the comma Spirilla in the harbour water of Marseilles after a period of eighty-one days, and that he himself has proved them to be still alive in different kinds of water over periods of time varying from twenty-four hours to thirty days. From my experiments it appears that the behaviour of the Spirillum in different waters is largely dependent upon the source from which they are obtained, but that under favourable circumstances a large amount of multiplication may take place when they are introduced into sewage

of the composition recorded, their behaviour in this medium presenting a very marked contrast to that in deep well and filtered Thames water.

Experiments with Finkler-Prior's Comma Spirillum.

Similar experiments to those described above were also made with this organism. These experiments show that although it possesses such far greater vital activity in gelatine cultures than Koch's comma Spirillum, yet when introduced into different kinds of water, including sewage, its vitality is so rapidly lost that in no single instance have I been able to demonstrate its presence even after twenty-four hours, still less to obtain evidence of any multiplication in these media. In fact so perishable does this organism appear to be when placed in water, that I have very frequently been unable to demonstrate its presence even on the day of inoculation, whilst with the *Bacillus pyocyaneus* I have never failed in this manner, and with the comma Spirillum only rarely. The following results will serve to illustrate how rapidly this organism loses its vitality in waters of various kinds:—

Finkler-Prior's Spirillum in Distilled Water.

Number of Colonies obtained from 1 c.c.

	1st Day.	3rd Day.	5th Day.	6th Day.	20th Day.
No. 1	12,000	0	0	—	—
„ 2	4,500	0	—	0	0

Finkler-Prior's Spirillum in Deep Well-water and Filtered Thames Water.

Number of Colonies obtained from 1 c.c.

		1st Day.	2nd Day.	4th Day.	5th Day.	9th Day.
No. 1	Deep well-water.	300	0	0	—	—
„ 2		300	0	0	—	—
„ 3		300	0	0	—	—
„ 4		17,450	0	—	0	0
„ 5		17,450	0	—	0	0
„ 6	Filtered Thames water	12,000	0	—	0	0
„ 7		12,000	0	—	0	0

Note.—Nos. 3, 5, and 7 were placed in the incubator.

Finkler-Prior's Spirillum in Sewage.

Number of Colonies obtained from 1 c.c.

	1st Day.	2nd Day.	4th Day.	5th Day.	9th Day.
No. 1	390	0	0	—	—
„ 2	390	0	0	—	—
„ 3	390	0	0	—	—
„ 4	10,750	0	—	0	0
„ 5	10,750	0	—	0	0

Note.—Nos. 3 and 5 were placed in the incubator.

From the differences which these three organisms present in their behaviour in water, and in different kinds of water, it is evident how fallacious must be any conclusions as to the vitality of pathogenic micro-organisms in general, more especially when such conclusions are based upon observations made with organisms which are the natural inhabitants of natural waters, as has often hitherto been the case. It is obvious that each individual organism must be made the subject of separate investigation as to its vitality. To render such investigations complete, it is necessary also that the organisms under examination should be taken from different sources, as from the experiments which I have quoted in the case of the comma Spirilla it is evident that an initial weakness of the growth from which the inoculation is made renders the organisms less capable of withstanding the conditions of the experiment. It would also appear probable that this initial difference in vitality may be the cause of some of the many discrepant results which have been obtained by different observers in the study of antiseptic action. That there exists a difference in resisting power according to the virulence or initial vitality of the organisms themselves has already been drawn attention to by Dr. Klein ("Micro-organisms and Disease," 1886).

In the following table the chemical composition of the sewage and other waters employed in the above experiments is recorded:—

Results of Analysis expressed in parts per 100,000.

	Total solid matter.	Organic carbon.	Organic nitrogen.	Ammonia.	Nitrogen as nitrates and nitrites.	Chlorine.	Hardness.
Sewage No. 1	60·20	2·350	1·387	2·200	0	8·5	—
„ „ 2	84·60	7·550	4·210	3·500	0	9·6	—
Deep well-water No. 1....	43·44	0·027	0·010	0	0·446	2·5	27·2
No. 2....	42·34	0·023	0·007	0	0·442	2·5	27·9
Filtd. Thames water.....	26·42	0·111	0·021	0	0·202	1·6	17·4

In conclusion I have to express my indebtedness to my wife for the great assistance which I have received from her in the most laborious task of estimating the colonies on the gelatine plates, amounting to nearly 1000 in number, which this investigation has entailed.

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